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EDI™ Human Growth Hormone ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Growth Hormone Level in Serum

REF **KT 819** EU: IVD CE Σ 96 +2 +8 °C

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human growth hormone (HGH) level in serum. This assay is useful as an aid for diagnosis and treatment of disorders involving the anterior lobe of the pituitary gland.

SUMMARY OF PHYSIOLOGY

Growth hormone (GH) is a protein-based poly-peptide hormone. It stimulates growth and cell reproduction and regeneration in humans and other animals. It is a 191-amino acid, single-chain polypeptide hormone that is synthesized, stored, and secreted by the somatotroph cells within the lateral wings of the anterior pituitary gland. Growth hormone is used clinically to treat children's growth disorders and adult growth hormone deficiency. In recent years, replacement therapies with human growth hormones (HGH) have become popular in the battle against aging and weight management. Reported effects include decreased body fat, increased muscle mass, increased bone density, increased energy levels, improved skin tone and texture, increased sexual function and improved immune system function. At this time HGH is still considered a very complex hormone and many of its functions are still unknown.

Growth hormone-releasing hormone and somatostatin released by neurosecretory nuclei of the hypothalamus into the portal venous blood surrounding the pituitary are the major controllers of GH secretion by the somatotropes. HGH is synthesized and secreted from the anterior pituitary gland in a pulsatile manner throughout the day; surges of secretion occur at 3- to 5-hour intervals. The plasma concentration of GH during these peaks may range from 5 to 45 ng/mL. The largest and most predictable of these GH peaks occurs about an hour after onset of sleep. Otherwise there is wide variation between days and individuals. Nearly fifty percent of HGH secretion occurs during the third and fourth REM sleep stages. Between the peaks, basal GH levels are low, usually less than 5 ng/mL for most of the day and night. Additional analysis of the pulsatile profile of GH described in all cases less than 1 ng/ml for basal levels while maximum peaks were situated around 10-20 ng/mL. A number of factors are known to affect HGH secretion, such as age, gender, diet, exercise, stress, and other hormones. Young adolescents secrete HGH at the rate of about 700 µg/day, while healthy adults secrete HGH at the rate of about 400 µg/day.

The most common disease of GH excess is a pituitary tumor composed of somatotroph cells of the anterior pituitary. These somatotroph adenomas are benign and grow slowly, gradually producing more and more GH. In children, growth failure and short stature are the major manifestations of GH deficiency, with common causes including genetic conditions and congenital malformations. Adults with GH deficiency present with non-specific problems including truncal obesity with a relative decrease in muscle mass and, in many instances, decreased energy and quality of life. Diagnosis of GH deficiency involves a multiple-step diagnostic process, usually culminating in GH stimulation tests to see if the patient's pituitary gland will release a pulse of GH when provoked by various stimuli.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human growth hormone in serum. The assay utilizes the two-site “sandwich” technique with two selected HGH-specific monoclonal antibodies that bind to different epitopes of human growth hormone.

Assay standards, controls and patient serum samples containing HGH are added directly to microtiter wells of microplate that was coated with streptavidin. Simultaneously, a biotinylated antibody and a horseradish peroxidase-conjugated antibody are added to each well. After the first incubation period, the wall of microtiter well captures the biotinylated antibody as well as an immunocomplex in the form of “streptavidin – biotin-antibody – HGH – HRP-antibody”. Unbound proteins as well as unbound HRP conjugated antibody in each microtiter well are removed in the subsequent washing step. The well is incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the HGH on the wall of the microtiter well is directly proportional to the amount of HGH in the sample. A standard curve is generated by plotting the absorbance versus the respective HGH concentration for each standard on Point-to-Point or 4-Parameter plot. The concentration of human HGH in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature.
Reagents from different kit lot numbers should not be combined or interchanged.

1. **Streptavidin Coated Microplate (Cat. No. 10040)**
One microplate with 12 x eight strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **HGH Tracer Antibody (Cat. No. 30409)**
One vial contains 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-HGH tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **HGH Capture Antibody (Cat. No. 30410)**
One vial contains 0.6 mL concentrated biotinylated anti-HGH capture antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. Tracer Antibody Diluent (Cat. No. 30017)
One vial contains 12 mL ready-to-use buffer. It should be only used for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. ELISA Wash Concentrate (Cat. No. 10010)
One bottle contains 30 mL of 30 fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. ELISA HRP Substrate (Cat. No. 10020)
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. HGH ELISA Stop Solution (Cat. No. 30357)
One bottle contains 12 mL of acidic solution of hydroxide chloride. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

8. HGH Standards (Cat. No. 30411 – 30417)
Seven vials each contain lyophilized human HGH in a bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration for each standard.** All the standards should be reconstituted with DI-water and stored at -20°C or below after the first use with up to 3 freeze-thaw cycles.

9. HGH Controls (Cat. No. 30418 – 30419)
Two vials each contain lyophilized human HGH in a bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be reconstituted with DI-water and store at -20°C or below after the first use with up to 3 freeze-thaw cycles.

SAFETY PRECAUTIONS
The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Hydroxide chloride may cause severe irritation and damage on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Wear laboratory gloves, eye glasses, etc. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, and 1000 µL etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 405 nm and 450 nm.

SPECIMEN COLLECTION
Only 50 µL of human serum is required for HGH measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected with Red-top Vaccutainer and separate the serum from cells by centrifugation (850 – 1500xg for 10 minutes). **Serum samples should be stored at –20ºC** if the assay is not to be performed within 5 hours. Avoid more than three freeze-thaw cycles of specimen. Do not use grossly hemolyzed, icteric or lipemic samples.

SPECIMEN SHIPMENT
Collected serum samples should be shipped to designated laboratory in frozen condition with dry ice.

ASSAY PROCEDURE
1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate (Cat. 10010)must be diluted to working solution prior use. Please see REAGENTS section for details.

(3) Reconstitute all assay standards (Cat. 30411-30417) and controls (Cat. 30418-30419) by adding **0.5 mL** of deionized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use. These reconstituted standards and controls must be stored at -10°C or below. Do not exceed 3 freeze-thaw cycles.

(4) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	C II
B	STD 1	STD 5	C II
C	STD 2	STD 6	SAMPLE 1
D	STD 2	STD 6	SAMPLE 1
E	STD 3	STD 7	SAMPLE 2
F	STD 3	STD 7	SAMPLE 2
G	STD 4	C I	
H	STD 4	C I	

(5) Prepare working Tracer Antibody and Capture Antibody mixture by 1:21 fold dilution of the HGH Tracer Antibody (Cat. 30409) and the HGH Capture Antibody (Cat. 30410) with the Tracer Antibody Diluent (Cat. 30017). For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with the addition of 50 µL of Tracer Antibody and 50 µL Capture Antibody in a clean test tube or vial. Following is a table that outlines the relationship of strips used and antibody mix prepared.

Strip no.	Tracer Antibody Diluent	Tracer Antibody	Capture Antibody
1	1 mL	50 µL	50 µL
2	2 mL	100 µL	100 µL
3	3 mL	150 µL	150 µL
4	4 mL	200 µL	200 µL
5	5 mL	250 µL	250 µL
6	6 mL	300 µL	300 µL
7	7 mL	350 µL	350 µL
8	8 mL	400 µL	400 µL
9	9 mL	450 µL	450 µL
10	10 mL	500 µL	500 µL
11	11 mL	550 µL	550 µL
12	12 mL	600 µL	600 µL

Note: this antibody mix should be freshly prepared right before running the assay.

3. Assay Procedure

(1) Place a sufficient number of streptavidin coated microwell strips (Cat. 10040) in a holder to run HGH standards, controls and unknown samples in duplicate.
(2) Add **25 µL** of HGH standards, controls and patient serum samples into the designated microwell.
(3) Add **100 µL** of above antibody mixture to each well.
(4) Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(5) Incubate plate at room temperature for **1 hour**.
(6) Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(7) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(8) Cover the plate with one new plate sealer and also with aluminum foil to avoid exposure to light.
(9) Incubate plate at room temperature for **15 minutes.** *(This incubation period may be reduced to 8 – 10 min if a lower OD reading is demanded to fit to the plate readers specification.)*
(10) Remove the foil and plate sealer. Add 100 µL of ELISA Stop Solution (Cat. 30357) into each of the wells. Mix gently.
(11) Read the absorbance at **405 nm** and **450 nm** within 10 minutes in a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The human growth hormone concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformations are used, sample with corrected absorbance between the 2nd standard and the next highest standard should be calculated by the formula:

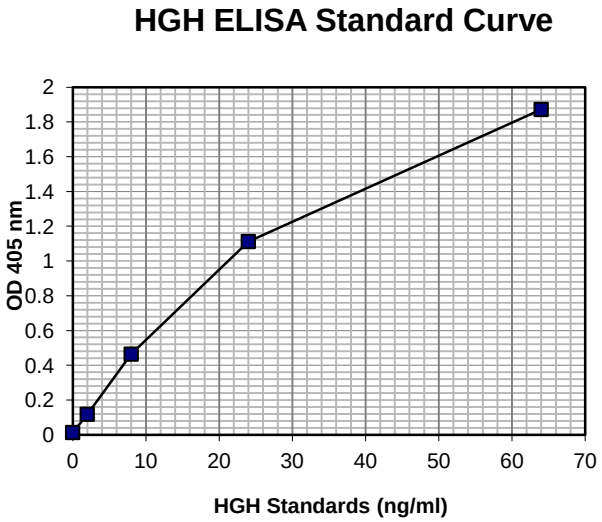
$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2nd STD)}} \times \text{Value of the 2nd STD}$$

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from HGH ELISA are represented. It is recommended to use two standard curves at OD 405 nm and 450 nm to calculate test results. **This curve should not be used in lieu of standard curve run with each assay.**

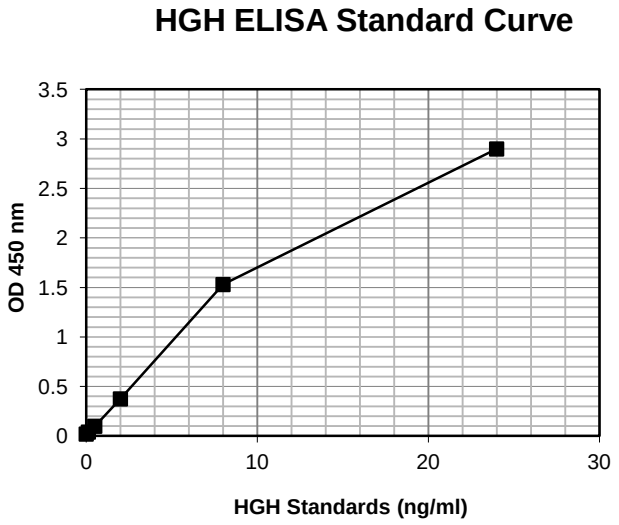
(1) **Example standard curve at OD 405 nm:** this curve should be used for routine sample measurement. It is recommended for reporting serum HGH level above 2 ng/mL. For sample HGH level above 64 ng/mL, it is recommended that the sample should be first diluted in an appropriate factor with a protein buffer matrix and then measured.

Well I.D.	OD 405 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.013 0.014	0.013	0.000	
2 ng/mL	0.120 0.116	0.118	0.105	
8 ng/mL	0.474 0.454	0.464	0.451	
24 ng/mL	1.088 1.136	1.112	1.099	
64 ng/mL	1.848 1.896	1.872	1.859	
Control I	0.075 0.074	0.074	0.061	1.17 ng/mL
Control II	0.268 0.281	0.275	0.262	4.71 ng/mL



(2) Example standard curve at OD 450 nm: this curve should be used if a highly sensitive HGH assay is required.

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.019 0.020	0.020	0.000	
0.125 ng/mL	0.039 0.040	0.040	0.020	
0.5 ng/mL	0.098 0.101	0.099	0.079	
2.0 ng/mL	0.382 0.367	0.375	0.355	
8.0 ng/mL	1.548 1.513	1.530	1.510	
24.0 ng/mL	2.885 2.913	2.899	2.879	
Control I	0.224 0.234	0.229	0.209	1.21 ng/mL
Control II	0.879 0.928	0.903	0.883	4.74 ng/mL



EXPECTED VALUES

Seventy-three normal adult sera were measured with this HGH ELISA. The cut-off for this normal group of population is 7.0 ng/mL.

It is highly recommended that each laboratory should establish their own normal range for HGH based on local populations.

LIMITATION OF THE PROCEDURE

1. For sample values reading greater than the value of the highest standard, it is recommended to re-assay samples with dilution.
2. Storing samples at refrigerated condition may cause significant degradation of HGH into small fragments.
3. Bacterial or fungal contamination of plasma specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay must include kit controls. We recommend that all assays include the laboratory's own human serum-based HGH controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity (LoD)

The sensitivity (LoD) of the HGH ELISA as determined by the apparent concentration of 3 standard deviation above the average of the zero standard on 10 duplicate determination of zero standard is approximately 0.087 ng/mL.

High Dose "hook" effect

This assay has showed that it did not exhibit any high dose "hook" effect up to 1,000 ng/mL.

Precision

The intra-assay precision is validated by measuring two controls samples in a single assay with 20 replicate determinations.

Mean HGH Value (ng/mL)	CV (%)
1.09	3.9
4.63	4.0

The inter-assay precision is validated by measuring two control samples in duplicate in 12 individual assays.

Mean HGH Value (ng/mL)	CV (%)
1.21	6.2
4.52	4.6

Linearity

Two human serum samples were diluted with zero standard matrix and assayed. The results in the value of ng/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	42.3	-	-
	1:2	20.6	21.2	97
	1:4	10.2	10.6	96
	1:8	4.61	5.29	87
2	Neat	21.7	-	-
	1:2	11.1	10.9	102
	1:4	4.7	5.4	87
	1:8	2.1	2.7	78

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. Leung KC, Howe C, Gui LY, Trout G, Veldhuis JD, Ho KK (October 2002). "Physiological and pharmacological regulation of 20-kDa growth hormone". *Am. J. Physiol. Endocrinol. Metab.* **283** (4): E836–43.
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3. Quabbe HJ; Ramek W, Luyckx AS (1983). "Growth hormone, cortisol and glucagon concentration during plasma free fatty acid depression. Different effects of nicotinic acid and an adenosine derivative". *J Clin Endocr Metab* **57** (2): 410–414.

Short Assay Procedure of HGH:

1. Add **25 µL** of standards, controls and patient plasma samples into the designated microwell.
1. Add **100 µL** of Diluted Tracer-/Capture-Antibody mixture into each well.
2. Incubate **1 hour** at RT.
3. Wash each well 5 times.
4. Add **100 µL** of ELISA HRP Substrate into each of the wells.
5. Cover and incubate plate at room temperature for **15 minutes**.
6. Add **100 µL** of ELISA Stop Solution into each of the wells.
7. Read the absorbance at OD 405 nm and 450 nm.

This product is developed and manufactured by
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Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

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